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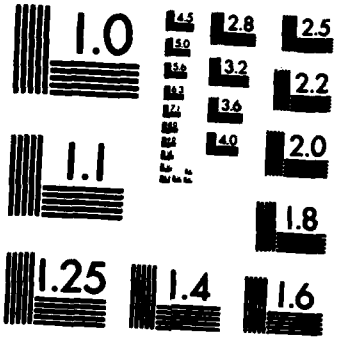


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20 The Second International Conference on Water and Ions was held in Bucharest, Romania, on September 6-11, 1982. This conference was sponsored by UNESCO's Biophysics Cooperation and The Romanian National Council for Science and Technology. In addition, some travel funds for American Scientists were provided by The Office of Naval Research and other agencies. Specifically seven Americn Scientists, partially supported by ONR funds, presented nine papers. Those reports included studies in cryobiology, hydration dependent phase transitions in membrane, proton transport in membrane bounded systems, selective ion accumulation in cellular organelles without membranes, and the physical properties of water and ions in biological and model systems.

The conference was well attended by a broad cross-section of international participants. Numerous papers were given on bound water in biological systems. New applications of neutron scattering techniques to the study of the diffusive motion of water molecules in biological and model systems were presented. Considerable time was devoted to the role of calcium in bioprocesses. Reports related to the transport of ions in membranes systems were in significant numbers.

It was announced that the Third International Conference on Water and Ions in Biological Systems will be held in Bucharest in the Fall of 1984.

Final Report for ONR Grant #N00014-82-G0099

Bucharest, Romania, September 6-11,1982

Principal Investigator

Carlton F. Hazlewood, Ph.D.

The Second International Conference on Water and Ions in Biological Systems was held in Bucharest, Romania, on September 6-11, 1982. Seven American Scientists were supported in part by this grant and each attended, presented contributions at the conference and submitted manuscripts for publication. In this report I will present a brief summary of the conference, a summary of the American contributions, and some conclusions concerning the conference.

OVERALL SUMMARY OF THE CONFERENCE. Sixteen symposia were held of fifteen subject areas which are as follows:

1. Interaction in ternary systems: water-ions-biopolymers
2. Organization and function of water in the native conformation of macromolecules
3. Hydrophobic interactions; their role in the organization of supramolecular structures
4. Water and ions in heterogenous systems

5a. Water and solute transport (part 1)
5b. Water and solute transport (part 2)

6. Role of water and ions in membrane structure and function
7. Proton translocation and bioenergetic phenomena
8. Calcium and the regulation of cellular processes
9. Heavy water effects in biosystems
10. Cryobiology
11. Physical techniques in the study of water in biological systems
12. Water and ions in muscle
13. Water and drug action, including anaesthetics
14. Ionic channels
15. Water and ions in evolution of biological systems

Eleven poster sessions were held concerning the following general topics:

1. Structure of water: bulk water, water in interfacial systems supercooled water, ice
2. Ternary systems: water - ions - biopolymers
3. Techniques providing information about the dynamics of molecular motions in water
4. Water, ions and bioenergetics
5. Water and ion transport in biological systems
6. Calcium and other divalent cations in biological systexs
7. Water and ions in the genesis and propagation of nerve impulse
8. Water and ions in the molecular mechanism of drug action
9. Fundamental and applied cryobiology
10. Water properties and ecological problems. Desalination of water
11. Water and ions: general aspects

The conference was well attended with a broad cross-section of international participants. Numerous papers were given on bound water in biological systems. The number of scientists which are proposing the existence of a large fraction

of cellular water that exists in a physical state different from ordinary water has increased remarkable (i.e., compared to the First International Conference on Water and Ions held in Bucharest, June 1980).

One symposium paper (H.E. Rorschach; Houston, Texas) and one poster (S. Todireanu, Bucharest, Romania) on the use of neutron scattering techniques in the study of water in biological systems were presented. This technology is relatively new to the biological and biophysical community and was well received. Neutron scattering provides new information on the diffusive motion of water molecules in biological systems. Considerable time was devoted to the role of calcium in bioprocesses. Reports related to transport of ions in membrane systems were in significant numbers as well.

## SUMMARY OF THE CONTRIBUTIONS OF THE AMERICAN SCIENTISTS

The seven American scientists supported, in part, by ONR Grant #N00014-82-G0099 and the titles of their presentation follow:

| | | |
|---|---|---|
| 1. | Dr. Harvey Bank<br>Medical Univeristy of South Carolina<br>Charleston, South Carolina | "Freezing: A Tool for the Preservation and Separation of Islets of Langerhans" |
| 2. | Dr. John Crowe<br>University of California at Davis<br>Davis, California | "Inhibition of Hydration Dependent Phase Transitions in Biological Membranes by a Carbohydrate" |
| 3. | Dr. Walter Drost-Hansen (2 papers)<br>University of Miami<br>Miami, Florida | "Anomalous Volume Properties of Vicinal Water and Some Recent Thermodynamic (DSC) Measurements Relevant to Cell-Physiology"<br><br>"Role of Vicinal Water in Cellular Evolution" |
| 4. | Dr. Raj K. Gupta<br>Institute for Cancer Research<br>Philadelphia, Pennsylvania | "Noninvasive NMR Probes of Intracellular Free Na and Mg Ions" |
| 5. | Dr. Carlton F. Hazlewood<br>Baylor College of Medicine<br>Houston, Texas | "Dynamic Interactions of Water Molecules, Inorganic Ions and the Chromatin Structures in Isolated Thymus Nuclei" |
| 6. | Dr. H.M. Rhee<br>Oral Roberts University<br>Tulsa, Oklahoma | "Differential Cation Transport in Functionally Different Canine Cardiac Tissues" |
| 7. | Dr. Elizabeth Simons<br>Boston University School of Medicine<br>Boston, Massachusetts | "$H^+$ Gradient Changes: Their Measurement and Their Significance in Cell Stimulation |

The following are summaries of the work presented by each of the above mentioned participants.

1. HARVEY BANKS: This study reveals that isolated islets of Langerhans (rat) can be frozen and thawed with little alteration in their physiology or morphology. The critical freezing parameters were found to be related to the type of cryoprotective agent used. For example the time and temperature of exposure to $Me_2SO$, the rate of cooling, the temperature at which dilution from the freezing medium occurs, and the presence of serum during the dilution procedures. When the freezing parameters are optimized, between 75 and 95% of the islets survived as measured by net glucose sensitive insulin release.

Banks reports having established an "optimal" cooling velocity in the presnence of 1M $Me_2SO$, a number of other variables were tested including time of exposure to $Me_2SO$ and dilution procedures. For such rapid cooling rates, maximal functional viability was obtained for islets exposed to 1M $Me_2SO$ at $4^o$ for 5 min. prior to freezing. Exposure times shorter than 1 min. or longer than 80 min. resulted in poor functional viability after freezing. The islets, when frozen in the presence of the culture medium containing $Me_2SO$, released 1½ times more insulin than did islets frozen in phosphate buffered saline containing $Me_2SO$. Banks reports a five-fold increase in total glucose-dependent insulin release compared to islets diluted at $37^o$ in the same medium with serum. Long term storage experiments showed no alteration in functional viability for islets stored at $-196^o$ for up to 1½ years. Banks techniques are compared with others.

2. J.H. CROWE AND L.M. CROWE: It was reported that as the water content of the membranes of the sarcoplasmic reticulum (S.R. vesicles) is decreased, there is a decline in the equilibrium freezing point until a limiting water content of 0.28-0.32 grams of $H_2O$/gram of dry solids is reached, below which water cannot be frozen. This non-freezable water content was measured using calorimetric methods. This group has developed a simple procedure for measuring thermal activity in a membrane sample during dehydration that permits simultaneous measurement of water content.

These workers have also used NMR techniques to evaluate phase behavior of phospholipids in a dynamic manner. In addition, they have utilized freeze fracture techniques and have confirmed that membrane phospholipids do undergo phase transitions which leads to complex crystal formation during dehydration.

3. W. DROST-HANSEN (2 papers): Recent measurements of the density of water in a porous silica gel strongly suggest that the density of vicinal water ($\rho_{vw}$) is notably lower than that of bulk water ($\rho_o$), consistent with the notion of enhanced structuredness of vicinal water. Recently, Etzler has provided the first semi-quantitative, satistical-mechanical model of vicinal water; the model allows an estimate to be made of the density of vicinal water in good agreement with the observed value. In view of the relatively large density difference ($\rho_o - \rho_{vw}$), it is suggested that pressure effects (even at rather low pressures may significantly affect the extent and possibly the nature

of vicinal water. Tentative evidence for such pressure effects will be discussed. Anomalous thermal expanison coefficient data and unexpected values for the temperature of maximum density were also presented.

Recent Differential Scanning Calometry (DSC) measurements were discussed. Drost-Hansen reported that his studies have confirmed the "Paradoxical Effect", i.e. the relative independence of vicinal water on the specific chemical nature of the adjacent solid interface and the existence of vicinal water more or less independently of the concentration and nature of dissolved electrolytes up to 1 molar concentration (thus also ruling out any electrical double-layer involvement in vicinal water structure.) (This subject was discussed at some length and remained controversal--that is, no consensus was reached.) The temperatures of the thermal transition ($T_k$) in vicinal water were observed to be invariant for surfaces as different as quartz, diamond and polystyrene. Finally, the specific heat of vicinal water was found in these measurements to be 1.25 ± 0.05 cal/$^oC$ gram in good agreement with our earlier results.

The role of the thermal transitions of vicinal water in cell-physiology were reviewed.

3. W. DROST-HANSEN (2nd paper): Vicinal water (i.e. water modified by proximity to interfaces) constitutes an important component of cell-associated water. The properties of vincinal water (v.w.) differ from those of the bulk; for instance, the heat capacity ($C_{p(uw)}$) is about 20% greater than the bulk value; the viscosity is notably larger (and may be shear-rate dependent); vicinal water enhances the condentration of structure-breaking ions (for instance $K^+$) over the concentration of structure-makers ($Na^+$) and the thermal expansion coefficient for vicinal water, $\alpha = (1/V)\ (dV/dT)_p$ is larger than the bulk value. However, most notable among the unusual properties of vicinal water (v.w.) is the occurrence of thermal transitions at discrete, narrow-temperature ranges. Thus, relatively abrupt changes occur in the properties of v.w. near 15$^o$ C, 30$^o$ C, 45$^o$ C, and 60$^o$ C. To be referred to as $T_1$, $T_2$, $T_3$, and $T_4$, or in general, $T_k$. Vicininal water has existed since liquid water was first formed on earth. In the process of evolution of the planet--and in the process of evolution of life in particular--vicinal water has been an "invariant". Life evolved in the presence of--and dependent on--vicinal water. In view of the ion distribution data referred to above, it may be assumed that "unusual" solute distribution must have occurred in proto-cells and early cell forms. It is conjectured that active transport across membranes in more complex cells evolved as a means of enhancing the $K^+/Na^+$ concentration effect and later found use as a general transport mechanism.

4. R.K. GUPTA, A.B. KOSTELLOW AND G.A. MORRILL: These workers report that a sizable fraction of sodium (by $^{23}Na$ NMR) is NMR invisible due to immolization and slow exchange resulting from binding and/or compartmentilization. Furthermore, these workers report that the predominately free fraction of rapidly exchanging Na increases during ovulation and early cleavage (study conducted with oocytes of Rana Pipiens). The increase in the NMR visibile $^{23}Na$ during the first meiotic division was found to coinside with the disappearance of Na, K-ATPase activity, suggesting an inverse relationship between the level of free $Na^+$ and active $Na^+$ transport.

5. C.F. HAZLEWOOD: A plenary lecture was given which attempted to summarize the findings on water molecules by three technologies: Nuclear magnetic resonance, dielectric relaxation, and neutron scattering. This report briefly summarizes some 10 years of collaborative research with Dr. H.E. Rorschach (Dept. of Physics, Rice University, Houston, Texas). Briefly, the reduction in diffusive motion observed in most biological systems cannot be accounted for by compartmentalization and/or cellular obstructions. Through the use of neutron scattering techniques, it has been possible to measure diffusive motion in a spacial domain short compared to inter-macromolecular distances within the cell. The findings demonstrate that both translational and rotational motions of water molecules are reduced in living cells and these reductions appear to be due to changes in the intrinisic properties of water (i.e., rather long-range interactions of macromolecular surfaces with water and not due to simple increases in the diffusive path length caused by obstructions). The neutron scattering data compare well to the relaxation time ($T_1$) determined by NMR techniques.

M. KELLERMAYER (Medical University of Pecs, Pecs, Hungary) and C.F. HAZLEWOOD: This collaborative work is directed at determining whether or not isolated fragments of protoplasm (e.g., organelles free of membrane structures) can selectively accumulate ions and retain water. Bovine thymus lymphocytes were isolated and treated with detergents ending with membraneless nuclei. The general morphology of the nucleus was maintained so long as the nuclei were maintained in sucrose buffer containing $Ca^{++}$ and $Mg^{++}$ (i.e., no monovalent ions such as $K^+$ and $Na^+$). The presence of monovalent ions caused dramatic changes in the morphology of the isolated nuclei. Furthermore, the amount of water retained by the isolated (membraneless) neuclei ranged between 64% and 78% (after exposure to 100,000 g forces) depending on the monovalent ion concentration of the buffer. It was also observed that $K^+/Na^+$ selectivity could be maintained depending on the detergent used.

6. H. RHEE: Quabain inhibitable, active uptake of $^{86}Rb^+$ was compared in seven functionally different tissues of canine heart. The highest active transport of $Rb^+$ was noted in the Purkinje fiber (8.4 nmoles/mg tissue, wet weight) and the least in the ascending aorta (0.8 mmoles/mg). The capacity of $Rb^+$ active transport in impulse conducting Purkinje fiber was significantly ($P < 0.001$) greater than the active $Rb^+$ uptake in the contractile ventricular muscle. This observation is consistent with the fact that $I_{50}$ (half-maximum inhibition) for ouabain in the Purkinje fiber is significantly lower than the $I_{50}$ value obtained from the ventricular muscle. However, there was a greater inhibition of transport adenosinetriphosphatase prepared from the ventricular muscle in vitro in comparison to the enzymatic activity obtained from the Purkinje fiber fromthe same animal. Therefore, it can be concluded that the well-known differential electrophysiological sensitivity to ouabain in these two functionally different tissues cannot account totally for the difference in the sensitivity of active transport of monovalent cation in this species.

7. E.R. SIMONS, N.E. NORMAN AND D.B. SCHWARTZ: Using the flourescence of certain cationic cyamine dyes as an indication of cellular resting potentials, these workers have monitered changes in flourescence as a function of pH and the concentration of substances such as thrombin, digitonin, and nigericin. The work reported was conducted on human platelets. In experiments utilizing

9-aminoacridine as the flourescent probe, they demonstrated that platelet $pH_i$ (cytoplasmic pH) rises upon thrombin stimulation. The maximum rise in $pH_i$ (in response to thrombin), was lower than that attained when the transplasma-membrane pH gradient is fully collapsed with 80 μM digitonin (i.e., when $pH_i = pH_{out} = 7.4$). The methods reported in this work allow for the measurement of transient changes in flouresence occurring on a time scale of seconds.

CONCLUSIONS OF THE CONFERENCE:

1. UNESCO has requested that the Third International Conference on Water and Ions be organized and held in Bucharest, Romania in the Fall of 1984.

2. The interaction of American scientists among themselves as well as with the scientists of some of the foreign countries have led to formal proposals for collaborative research.

3. A proceedings of the conference will be published by Plenum Press and preprints of all the papers reviewed in this report may be obtained from the authors.

4. Although many problems arise at international meetings, the benefits seem to outweigh the personal difficulties that arise. Several sound collaborative research efforts have arisen between American and foreign scientists. I personally feel that it is to the benefit of American biophysical science to continue to participate in this UNESCO program.